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Review

Use of magnetic techniques for the isolation of cells

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Abstract

Magnetic separation is an emerging technology using magnetism, sometimes in combination with conventional separation or identification methods, to purify cells, cell organelles and biologically active compounds (nucleic acids, proteins, xenobiotics) directly from crude samples. Several magnetic separation procedures have been developed to isolate target cells specifically. The purpose of this short review is to summarize various methodologies, strategies and materials which can be employed for the selection and separation of target cells with the help of magnetic field and thus to help the novices in this field to be able to orient themselves in vast amount of literature available. Immunomagnetic separations employing specific antibodies to label the target cells represent the most often used approach and are discussed in detail.

Keywords: Reviews; Magnetic separation; Immunomagnetic separation; Cells

Contents

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1. Introduction

For a long time, magnetism has stood out as an interesting and important driving force to separate magnetic from non-magnetic components of the mixture. Enrichment of low grade iron ore, removal of ferromagnetic impurities from large volumes of boiler water in both conventional and nuclear power plants or removal of weekly magnetic coloured impurities from kaolin clay are the typical examples of industrial applications of magnetic separations [1].

The applications of these techniques to biosciences have been restricted and of limited use up to the 1970s. The idea of using magnetic separation techniques in cell separation has enjoyed a resurgence of interest over the last decade. This has primarily been brought about by the development of new magnetic particles with improved properties for various cell separation procedures.

Magnetic separation of cells has several advantages in comparison with other techniques used for the same purpose. It permits the target cells to be isolated directly from crude samples such as blood, bone marrow, tissue homogenates, stool, cultivation media, food, water, soil etc. Compared to other more conventional methods of cell separation, magnetic separation is relatively simple and fast and in a way may be considered a sample enrichment step for further chromatographic and electromigratory analysis. The static magnetic field does not interfere with the movement of ions and charged solutes in aqueous solutions (at low flow-rates) as does the electric field. Furthermore, the large differences between magnetic permeabilities of the magnetic and non-magnetic materials can be exploited in developing highly selective separation methods. This high selectivity can be achieved with other separation methods only through extensive and costly instrumentation development [2]. The sheer forces associated with binding and elution are minimal compared to centrifugation or filtration increasing the yield of active cells. In general, the magnetic separation procedure is gentle, facilitating the rapid handling of delicate cells in an unfriendly environment. It also simplifies procedures such as change of buffer conditions and repeated washing steps. Unlike conventional flow cytometry methods, the selection of defined cell populations can easily be scaled up if large quantities of living cells are requested. The cells isolated by magnetic separation process are usually pure, viable and unaltered. High sample throughput and automation of routine purifications is only possible with magnetic separation with no need for elaborate protocols, expensive equipment or costly consumables. The whole separation process can be performed in the same tube running multiple samples simultaneously in a fraction of the usual time.

Several review papers can be found in the literature describing various aspects of magnetic cell separation. These papers are usually oriented on specific topics, such as the application of magnetic separation techniques in microbiology [3,4], immunomagnetic separation of cells using Dynabeads [5–8], cell separation with magnetic colloidal labels [9] or the application of carbohydrate coated magnetic beads for the isolation of cell that specifically express cell surface carbohydrate binding molecules [10]. The purpose of this short review is to summa-

rize various methodologies, strategies and materials which can be employed for the selection and separation of target cells with the help of magnetic field and thus to help the novices in this field to be able to orient themselves in vast amount of literature available.

2. Principle of magnetic separation techniques

Generally speaking, there are two types of magnetic separations when working with cells. In the first type, cells to be separated demonstrate sufficient intrinsic magnetic moment so that magnetic separations can be performed without any modification. There are only two types of such cells in the nature, namely red blood cells (erythrocytes) containing high concentrations of paramagnetic haemoglobin, and magnetotactic bacteria containing small magnetic particles within their cells. In the second type, one or more non-magnetic (diamagnetic) components of a mixture have to be tagged by a magnetic label to achieve the required contrast in magnetic susceptibility between the cell and the medium. The attachment of magnetic labels (mainly particles) is usually mediated by affinity ligands of various nature, which can interact with target structures on the cell surface. In the most often used approach antibodies (Abs) against specific cell surface epitopes are used, but other specific ligands can be employed, too (see later). The newly-formed complexes have magnetic properties and can be manipulated using an appropriate magnetic separator [1,11].

The separation process for the purification of target cells using magnetic labels and magnetic separators usually consists of the following three fundamental steps (independent of the scale of preparation): (1) the suspension containing the cells of interest is mixed with magnetic labels. Interaction of the target cells and the labels occurs during the incubation step (usually not longer than 30–60 min in laboratory scale). Then the magnetic complex formed is separated using an appropriate magnetic separator and the supernatant is discarded or used for another application. (2) The magnetic complex is washed several times to remove unwanted contaminants. In this form the selected cells with attached magnetic labels can be directly used e.g. for cultivation experiments. Alternatively, the cells can be disrupted and the cell content analyzed using variety of methods (chromatography, electrophoresis, PCR etc.). (3) For selected applications magnetic label has to be removed from the separated cells. Variety of detachment procedures exists (see later). After detachment magnetic label is removed from the suspension in a separator and free cells are ready for further applications and analyses using variety of methods.

Magnetic separation of cells is usually performed in a batch mode. From the point of view of chromatographic and electromigration methods these magnetic separation techniques can be considered to be the pre-separation ones. Nevertheless, separations using magnetically stabilized fluidized beds (MSFBs) belong among the standard fluidized-bed chromatography separation procedures [114,115]. Up to now there are only several examples where MSFBs and cells have been combined together [116].

3. Equipment necessary to perform magnetic separation of cells

Magnetic labels and magnetic separators are necessary to be able to perform efficient cell separation. Typical examples are given in the following sections.

3.1. Magnetic labelling agents

With the exception of magnetotactic bacteria and erythrocytes the cells to be isolated have to be magnetically labelled in order to be susceptible to magnetic treatment. Several strategies can be chosen, depending on the character of the target cells and the subsequent operations. Magnetic labelling can be performed with magnetic and superparamagnetic particles, magnetic colloids, magnetoliposomes or with molecular magnetic labels. In most cases the magnetic properties of the labels are caused by the presence of small particles of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3); in some cases also ferrite particles or chromium dioxide particles [12] have been used.

There is a constant discussion about the application of large (ca. 1 μm and more in diameter) and small (ca 50–200 nm) magnetic particles for cell

labelling. Particle size determines the physical behaviour and kinds of manipulations possible with a particular particle. Both types of these magnetic labels have been used successfully for many applications, but for a specific area one type of magnetic label is better. Regarding the kinetics of labelling cells, the colloidal labels (ferrofluids) are clearly superior as they react more quickly and require no mixing of the mixture. On positive selection (removal of the population of cells of interest for subsequent use), large particles usually form cages of magnetic material around positively selected cells and in many cases the magnetic particles have to be removed from the cell surface. Relatively large, dense particles tend to settle and therefore there is a need for agitation. On the other hand, for cells isolated with colloidal or molecular labels various manipulations can generally be undertaken immediately following isolation. Their affinity reactions do not depend on mixing, while simple diffusion and Brownian motion keep the magnetic solute uniformly distributed throughout the cell suspension without agitation. Regarding the potential of dislodging a labelled cell receptor, larger particles where one particle can make many receptor contacts would have an advantage. The magnetic separation of cells labelled with larger particles can be performed in a simple and cheap separator, while more expensive high gradient magnetic separators or other advanced systems have to be usually used for the separation of cells labelled with colloidal or molecular labels.

3.1.1. Magnetic and superparamagnetic particles

The diameter of magnetic particles used for cell separations is typically 1–5 μm, i.e., they are on the order of a cell diameter. Most of the particles used are superparamagnetic, that is they only exhibit magnetic properties in the presence of external magnetic field. They can be easily removed from a suspension with a simple magnetic separator. Since there is usually no magnetic remanence the particles are not attracted to each other and therefore they can be easily suspended into a homogeneous mixture in the absence of any external magnetic field [5]. Magnetic particles typically comprise fine grains of iron oxides dispersed throughout the interior of a polymer particle (in many cases of a monosized type), the surface chemistry of which can be modified to provide a range of different linking methods. Alternatively, silanized particles of magnetic iron oxides or magnetic porous glass can be used for the same purpose.

A number of particulate magnetic labels can be purchased commercially. Up to now in most applications monosized polymer particles marketed as Dynabeads (Dynal, Oslo, Norway) in various forms have been used. Dynabeads are prepared from monosized macroporous polystyrene particles which are magnetized by an in situ formation of ferrimagnetic material inside the pores. The reproducibility in the preparation of the particles can be ascribed to the method of "activated swelling" [7]. Dynabeads have diameters of 2.8 μm (Dynabeads M-280), 4.5 μm (Dynabeads M-450) and 5.0 μm (Dynabeads M-500). The Dynabeads M-450 particles have a surface area of 1–4 $m^2\ g^{-1}$, a density of 1.5 g cm^{-3} with an iron content of 20% (w/v) and the number of particles in 1.0 mg is $1.4 \cdot 10^7$ [8]. Polymer shell on the surface of the beads protects the target cells from the possible toxic exposure to iron. Both M-280 and M-450 carriers can be obtained in non-activated and tosyl-activated form. Coated Dynabeads with covalently immobilized streptavidin or primary and secondary Abs are also available.

Also other commercially available magnetic particles prepared by different procedures can be used successfully. A non-complete selection of these products can be found in the Table 1.

Magnetic beads for use in cell separation should fulfil some important criteria [13], i.e., they should be chemically stable and should not aggregate in the media used in cell separation, they should show very little magnetic remanence after having been subjected to the magnetic field, they should not bind to cells non-specifically, there should be very little leakage of the immobilized affinity ligand (Ab, antigen, lectin, carbohydrate) from the particles during storage, they should allow a fast and complete magnetic separation of the cells labelled with particles and of excess particles from the unlabelled cells, and they should be of a size which minimizes phagocytosis. Majority of the commercially available particles fulfils these requirements.

3.1.2. Colloidal magnetic labels

Colloidal magnetic labels (typical size is ca. 50–200 nm) are prepared by a variety of methods which

Table 1
A non-complete selection of commercially available magnetic and superparamagnetic particles used (or suitable) for the isolation of cells

Name	Diameter (μm)	Polymer composition/ surface modification	End groups and activation possibility	Immobilized antibodies	Other immobilized compounds	Manufacturer/ supplier
BioMag	~1	Silanization of iron oxides	–COOH, $–NH_2$	Secondary Abs, anti-CD Abs, anti-fluorescein Ab	Protein A protein G, streptavidin, biotin	PerSeptive Biosystems, Farmingham, MA, USA
Dynabeads M-280 Dynabeads M-450 Dynabeads M-500	2.8 4.5 5	Polystyrene	Tosyl-activated	Secondary Abs, anti-CD Abs, Abs against *E. coli* O157, *Salmonella Listeria*, *Cryptosporidium*	Streptavidin, oligo (dT)	Dynal, Oslo, Norway
Estapor	~1	Polystyrene	–COOH, $–NH_2$			Prolabo, Fontenay-sous-Bois, France
Iobeads	~1			Anti-CD Abs, secondary Abs	Avidin	Immunotech, Marseille, France
M 100 M 104 M 108	1–10	Cellulose	–OH			Scigen, Sittingbourne, UK
MagaBeads	3.2	Polystyrene	–COOH, $–NH_2$, epoxy	Secondary Abs	Streptavidin protein A, protein G	Cortex Biochem, San Leandro, CA, USA
Magne-Sphere	<1				Streptavidin	Promega, Madison, WI, USA
Magnetic beads	0.8	Latex			Streptavidin, protein A, protein G	ProZyme, San Leandro, CA, USA
Magnetic microparticles	1–2	Polystyrene	–COOH $–NH_2$		Protein A	Polysciences, Warrington, PA, USA
Magnetic particles	1	Polystyrene		Anti-digoxigenin Ab	Streptavidin	Boehringer, Mannheim, Germany
Magnetic particles	~1	Polystyrene				Bangs Labs., Fishers, IN, USA
MPG	5	Porous glass	$–NH_2$, hydrazide, glyceryl		Streptavidin, avidin	CPG, Lincoln Park, NJ, USA
Sera-Mag	1	Polystyrene	–COOH		Streptavidin	Seradyn, Indianapolis, IN, USA
SPHERO magnetic particles	Various (1–4.5)	Polystyrene	–COOH, $–NH_2$	Secondary Abs	Streptavidin, biotin	Spherotech, Libertyville, IL, USA
XM200 microsphere	3.5	Polystyrene	–COOH	Secondary Abs	Protein A	Advanced Biotechnologies, Epsom, UK

results in "flocks" composed of polymer (typically dextran, starch or protein) and magnetite and/or other iron oxide crystals. Molday and Mackenzie [14] described a procedure for the synthesis of superparamagnetic dextran nanospheres by precipitation of iron oxide in the presence of the polysaccharide. Alternatively superparamagnetic iron oxides (obtained by precipitation of ferric and ferrous salts in the presence of sodium hydroxide) are coated with polysaccharides or with synthetic polymers [15]. To such materials ligands (usually Abs, lectins, streptavidin or biotin) are coupled so they can be used for cell separation [16,17]. Using high gradient magnetic columns the labelled cells are easily separated. Later particles of larger diameter, having ferrofluid-like behaviour have become available. These magnetic colloid labels (ferrofluids) do not require the high magnetic gradients previously used for separation and, in fact, they separate in fields nearly an order of magnitude less then was being used.

Also magnetic particles isolated from magnetotactic bacteria can be successfully used. The particles (50–100 nm) are usually composed of magnetite covered by a stable lipid membrane. Particles can be easily activated and biologically active compounds can be immobilized [18,19].

An example of commercially available products is given in Table 2.

3.1.3. Magnetoliposomes

Magnetoliposomes are magnetic derivatives of ordinary liposomes prepared by incorporation of colloidal magnetic particles into the lipid vesicles. Magnetoliposomes are usually used as carriers for drug targeting [20] or for immobilization of membrane-bound enzymes [21]. When magnetoliposomes are associated with Abs they enable to label and/or to concentrate selectively the target cells [22–24].

3.1.4. Molecular magnetic labels

Lanthanides, especially erbium in the form of erbium chloride ($ErCl_3$), have been used for magnetic labelling of a variety of cells. Erbium ions have a high affinity for the external cell surface and preserve their exceptionally high atomic magnetic dipole moment (9.3 Bohr magnetoms) in various chemical structures [25,26]. The mechanism of Er^{3+} binding to the cell surface is mostly ionic, with many different Er^{3+} binding sites, such as carboxyl groups in glycoproteins, differing in affinity and binding capacity. The other well recognized lanthanide binding sites are the Ca^{2+} receptor sites on the cell wall [25].

Ferritin is a naturally occurring, soluble iron storage protein in mammals. It consists of a hollow protein shell (relative M_r 450 000) of approximately 13 nm in diameter, with a cavity of approximately 7 nm. Iron is deposited within the cavity in the form of a hydrous ferric oxide $5Fe_2O_3 \cdot 9H_2O$, in amounts which vary from zero to 4500 atoms of Fe(III) [27]. For magnetic modification of cells cationized horse spleen ferritin (ferritin coupled with *N,N*-dimethyl-1,3-propanediamine) exhibiting a net positive charge at pH 7.5 is usually used. Under these conditions the

Table 2
A non-complete selection of colloidal magnetic labels

Name	Diameter (nm)	Polymer composition	End groups and activation possibility	Immobilized antibodies	Other immobilized compounds	Manufacturer/ supplier
Ferrofluids	135, 175	Modified hydrophilic protein	–COOH, $-NH_2$	Secondary Abs	Streptavidin, protein A	Immunicon, Huntingdon Valley, PA, USA
MACS microbeads	50	Dextran	–OH	Secondary Abs, anti-CD Abs	Streptavidin, biotin	Miltenyi Biotec, Bergisch Gladbach, Germany
Magnetic nanoparticles	90–600	Starch, dextran, chitosan	–OH –COOH		Streptavidin, protein A, biotin	Micro-caps, Rostock, Germany
MagNIM	50, 250, 500		–COOH $-NH_2$	Secondary Abs, Ab against *E. coli* O157	Streptavidin, protein A	Cardinal, Santa Fe, NM, USA

cationized ferritin readily forms ionic bonds with the anionic sites on the cell membrane [2].

Magnetic derivatives of ferritin called magnetoferritin have also been prepared. A magnetic mineral was synthesized within the nanodimensional cavity of horse spleen ferritin using controlled reconstitution conditions. Transmission electron microscopy and electron difraction analysis, together with Mössbauer spectroscopic and magnetic measurements, indicated that the entrapped mineral particles are discrete 6–7 nanometer spherical single crystals, composed of ferrimagnetic iron oxide maghemite (γ-Fe_2O_3). The magnetization of the particles in magnetoferritin saturates at relatively low field strengths, with no hysteresis effects (magnetic remanence) and a molecular magnetic moment of about 13 000 Bohr magnetoms. The particles are thus superparamagnetic [1,28,29].

3.2. Magnetic separators

A variety of magnetic separators is available on the market starting with very simple concentrators for one test tube and ending with complicated fully automated devices. In many cases, especially when working with larger labels, very cheap laboratory-made magnetic separators can be used successfully [30]. Laboratory-scale magnetic separators can be sorted according to their construction, type of operation etc. In this review two basic types of separators will be distinguished, namely batch and flow-through ones. Short information about devices to perform magnetically stabilized fluidized bed chromatography will also be given.

3.2.1. Batch magnetic separators

In most cases isolation of magnetically labelled cells is performed in a batch mode, using commercially available laboratory scale magnetic separators (particle concentrators). Separators are usually made from strong rare-earth permanent magnets embedded in disinfectant-proof material. The racks are designed to hold various amounts of microtubes or tubes. Some of the separators have a removable magnet plate to facilitate easy washing of magnetic particles. Test tube magnetic separators enable to separate magnetic particles from volumes ranging approximately between 5 μl and 50 ml. It is also possible to separate cells from the wells of standard microtitration plates. Magnetic complexes from larger volumes of suspensions (up to approx. 500–1000 ml) can be separated using flat magnetic separators.

A selection of more sophisticated magnetic separators is also available. Immunicon (Huntingdon Valley, PA, USA) has developed a device that consists of a non-magnetic T-shaped frame into which two ferromagnetic wires, each bent in a loop shape are inserted for each separation to be performed. The "pins" are inserted into microtiter wells (or other vessels) located in the magnetic field. Magnetically labelled cells are collected on the "pins". The same company is also producing separators employing external gradient magnetic fields, based on the quadrupole and hexapole magnet configuration.

A non-complete selection of commercially available batch magnetic separators is given in Table 3.

3.2.2. Flow-through magnetic separators

Flow-through magnetic separators are characterized by the flow of the liquid and suspended cells through the separation system. These systems are usually more expensive and more complicated in comparison with batch separators, but for preliminary experiments simple devices can also be used [31].

Laboratory-scale high gradient magnetic separators (HGMSs) are in fact variants of industrial separators. Small columns loosely packed with fine magnetic grade stainless steel wool are placed between the poles of strong permanent magnets or electromagnets. Magnetically labelled cells are pumped through the column, labelled cells are retained on the steel wool, next the field is removed and cells are retrieved by flow and usually by gentle vibration of the column [16]. Commercially available systems are produced by Miltenyi Biotec, Bergisch Gladbach, Germany (MACS cell sorters).

Ferrography is a method of particle separation onto a glass slide based upon the interaction between an external magnetic field and the magnetic moments of the particles suspended in a free-flowing, open stream. The separated material is preserved on a glass slide in a form suitable for further routine cytological analysis [25,26,32]. A more sophisticated intrumentation, which evolved from ferrography, is called analytical magnetapheresis [2].

The continuous immunomagnetic sorter described

Table 3
A non-complete selection of commercially available magnetic separators working in a batch mode

Company	Designation of the separator	Description
Advanced Biotechnologies, Epsom, UK	·10/20 place separator ·Base separator	·Separator for 10 or 20 1.5-ml reaction tubes, two 15-ml tubes and one 50-ml tube ·For large scale isolations
Biometra Biomedizinische Analytik, Göttingen, Germany	Magnetic separator stand	Stand for 10 1.5-ml reaction tubes, two 15-ml tubes and one 50-ml tube
Boehringer, Mannheim, Germany	Magnetic particle separator	Separator for four 1.5-ml reaction tubes
CPG, Lincoln Park, NJ, USA	3-in-1 magnetic particle separator	Separator for eight 1.5-ml reaction tubes, one 15-ml tube and one 50-ml tube
Dynal, Oslo, Norway	·MPC-1, MPC-2, MPC-6 ·MPC-E-1, MPC-E-6, MPC-M	·Separators for 1, 2 or 6 test tubes ·Separators for 1, 6 and 10 microcentrifuge tubes of the Eppendorf type
Immunicon, Huntingdon Valley, PA, USA	·PS4100 ·XS4200	·"Pin" separator for the separation of cells from microtiter wells or similar vessels ·Quadrupole magnetic separator
Immunotech, Marseille, France	·Magnetic holder for 1 tube ·Magnetic holder for 4 tubes	Separators for 1 or 4 test tubes, respectively
Promega, Madison, WI, USA	MagneSphere magnetic separation stands	Different magnetic stands for 2 and 12 test tubes of different diameters
Quantum Magnetics, Madison, CT, USA	Versatile separator	Separator can accommodate multiple tubes and various sizes of tubes simultaneously
Sigris Research, Brea, CA, USA	MixSep	A device combining mixing and separation of magnetic particles

by Hartig et al. [33] is based on an electrophoresis counter-flow chamber. A mixture of magnetically labelled and non-labelled particles is injected into a continuously flowing chamber buffer. The particles pass the inhomogeneous magnetic field of the open-gradient electromagnet in two narrow streams. According to the magnetic moments, induced by the magnetic field, magnetically labelled particles are deviated and focused into a stream that is completely separated from the streams of the non-deviated particles.

Quadrupole magnetic separators have been studied by Chalmers et al. [34]. In this type of magnetic separator four magnetic "poles" focus the magnetic field around a central, cylindrical area. The quadrupole separator splits an inlet, magnetically labelled, cell stream into two outlets one of which contains mainly the magnetically labelled cells.

Baxter Healthcare (Deerfield, IL, USA) developed Isolex 300i magnetic cell separator which is now used for the large-scale selection of CD34+ cells. All cell preparation and washing is done automatically and the device represent a flexible platform for future applications in cell separation [35,36].

Examples of commercially available flow-through magnetic separators are given in Table 4.

3.2.3. Magnetically stabilized fluidized bed separators

These devices are usually composed of a column filled with an appropriate ferromagnetic packing materials (e.g., nickel spheres [116], magnetic poly-

Table 4
A non-complete selection of commercially available magnetic separators working in flow-through mode

Company	Designation of the separator	Description
Baxter Healthcare, Deerfield, IL, USA	Isolex 300i	An automated system for CD34+ cells isolation
Dr. Weber, Kirchheim-Heimstetten, Germany	Continuous immunomagnetic sorter CIMS11	Modular continuous sorter
Immunicon, Huntingdon Valley, PA, USA	HG4100	High-gradient magnetic separator
Miltenyi Biotec, Bergisch Gladbach, Germany	Various types of MACS separators	High-gradient magnetic separators
Quantum Magnetics, Madison, CT, USA	Flow cell separator	Separator made of rare earth magnets allowing one to insert a rectangular flow cell or stationary tube

acrylamide beads [117] or beads of κ-carrageenan incorporating magnetite [118]) which is placed in magnetic field. The field is typically applied co-linear with that of mobile phase by surrounding the column with a solenoid. Using a solenoid allows a researcher to vary magnetic field strength by varying applied current. The magnetic field improves performance of a fluidized bed separator by inhibiting axial motion of the packing material and reducing bed expansion thus allowing a magnetically stabilized fluidized bed separator to perform essentially the same as a packed bed but at significantly lower operation pressure drops [114,115].

4. Procedures to perform magnetic separation of cells

Magnetic separation of cells can be performed in one of the following formats. (1) Direct method. The affinity ligand is coupled to the magnetic particles, which are then added directly to the cells containing sample. During incubation the magnetic particles will bind the target cells which can be then recovered using a magnet. (2) Indirect method. In the first step, target cells are sensitized with a suitable primary affinity ligand. After incubation excess unbound affinity ligand is usually removed by washing the cells and then magnetic particles with immobilized secondary affinity ligand with the affinity for the first affinity ligand are added. The magnetic particles will bind the target cells which can be then recovered using a magnetic separator.

Another differentiation of magnetic separation techniques is based on the selection of the magnetically labelled cells. (a) Negative selection is a method by which a cellular subset is purified by removing all other cell types from the sample. Both the direct and indirect method are applied for negative selection. The negative selection of cells has the advantage that the purification process does not involve any direct contact with the cells to be isolated. This technique has its limitation. If the subsets of cells to be selected are present in a low concentration, negative selection may give low yield and purity due to non-specific loss of the cells to be isolated, or due to an insufficient removal of unwanted cells. (b) Positive selection. In this case, the target cells are isolated from the cell suspension. Both the direct and indirect method can be used. The magnetically labelled cell complexes separated can be further characterized directly, but in many cases it is necessary to remove larger magnetic particles from the positively selected cells after their isolation. (c) Depletion of cells. Depletion is a method by which one or more unwanted cellular subsets is removed from a cell suspension. Both the direct and the indirect procedure can be applied for this purpose. The indirect method is generally more efficient in removing unwanted target cells from a suspension [8].

In the next section magnetic separation of cells is

discussed from the point of view of affinity ligands used to label the target cells.

4.1. Immunomagnetic separations

Immunomagnetic separation (IMS) of prokaryotic and eukaryotic cells is described in a steadily increasing number of scientific articles and booklets [5–8,37]. The number of papers in databases such as PubMed, employing the principle of immunomagnetic separation, is approaching 1000 and is rapidly increasing.

IMS of cells implies the use of magnetic beads or magnetic colloids–antibody system causing the particles to be selectively attached to target cells when added to a cell suspension. After incubation, cells with attached magnetic particles (and also excess particles) are isolated with the help of an appropriate magnetic separator. Most often, a monoclonal antibody (MAb) is used for IMS, but in many cases polyclonal Abs are used successfully. IMS can be performed in all the formats mentioned above.

In the direct method the appropriate Ab is coupled to the magnetic particles, which are then added directly to the sample. Ideally, the Ab should be oriented with its Fc part towards the magnetic particle so that the Fab region is pointing outwards from the particle. Several procedures are available for direct binding of Abs:

- Adsorption of Abs on hydrophobic magnetic particles, especially those made of polystyrene (this technique is relatively often used while working with Dynabeads) [8,38].
- Covalent binding of Abs on activated magnetic particles (e.g., tosylactivated Dynabeads), or on magnetic particles carrying appropriate functional groups (e.g., amino, carboxy, hydroxy) using standard immobilization procedures [8,39].
- Secondary Abs (i.e., Abs against primary Abs) are immobilized first on magnetic particles and then primary Abs are bound. The secondary Abs may in this case function as a spacer and lead to a favourable orientation of primary Abs [5].
- Streptavidin immobilized on magnetic carriers binds biotinylated Abs [40,41].
- Protein A and protein G immobilized on magnetic particles bind the Fc region of IgG of most mammalian species leaving antigen-specific sites free [12].
- Magnetic carriers with immobilized boronic acid derivative reversibly bind Abs through the interaction with the carbohydrate units on the Fc part of Ab. By this method favourable orientation of the Abs to the carrier surface is obtained [7].
- Primary Abs tagged with oligo dA are coated on magnetic particles with immobilized oligo dT utilising the hybridisation between oligo dA and oligo dT homopolymers [42].
- Magnetic beads with attached DNA containing the *Escherichia coli* lac operator bind fusion proteins comprising the DNA-binding lac repressor [43].
- Magnetic carriers bearing hydrazide groups can be used for oriented immobilization of Abs via their carbohydrate moiety [24].

The indirect method is also used very often. In a first step, the cell suspension is incubated with primary Abs which bind to the target cells. Prior sensization of the target cells will ensure a proper orientation of the Abs and an optimal number of interaction possibilities between magnetic particles and cells. Not only purified primary Abs have to be used; crude Ab preparations or serum can be used, too [44]. After incubation, the unbound Abs are usually removed by washing. Thereafter, the magnetic particles with immobilized secondary Ab are added, permitting the beads to bind rapidly and firmly to the primary Abs on the target cells. Target cells–primary Ab complexes can be also captured by protein A or protein G immobilized on magnetic carriers [45]. Alternatively, primary Abs can be biotinylated or labelled with fluorescein and magnetic particles with immobilized streptavidin or anti-fluorescein Ab are used for capturing the target cells [46,47].

For most monoclonals, the indirect method is generally more efficient in removing target cells from a suspension. This is due to the fact that "free" Abs will find their target antigen more easily than Abs bound to magnetic particles. The indirect technique is the method of choice when a cocktail of MAbs is used. The indirect technique is also recommended when the target cell has a low surface

antigen density. The direct method usually requires less incubation time, and therefore it is faster than the indirect method. When MAbs of your choice are used with the secondary coated magnetic particles, the direct method requires less Ab then the indirect method. Also, the direct method is advantageous when one does not want to cover all antigen sites with Ab [8].

Both negative and positive selection of cells is used in IMS. 95–99% viability and purity of the positively isolated cells are typically achieved with a typical yield of 60–99%. Depletion efficiency often reaches 99.9% and leaves remaining cells untouched. The amount of beads and Abs required will generally be higher than for positive isolation. Sequentional depletions are markedly more efficient [8].

The positively selected cells may, in many cases, not show any interference from the larger magnetic particles and may also be analyzed with the particles attached on them. In some cases, however, it is necessary to remove larger immunomagnetic particles from the cells after their isolation. The detachment process can be performed in several ways:

- Detachment can, in some cases, be obtained by incubating rosetted cells overnight in cell culture medium. The mechanism most likely to be responsible for detachment is transient downregulation of the target surface antigen by the target cell. The detachment efficiency is further enhanced by mechanical forces such as firm pipetting flushing the suspension 5–10 times through a narrow tipped pipette. Magnetic particles will then be released from the cells and can be removed with a magnetic separator [8].
- Proteolytic enzymes can be used to release isolated cells from magnetic particles. Chymopapain has been shown to cleave selectively a segment of the CD34 molecule from human hematopoietic cells and thereby detach magnetic particles coated with Ab against CD34. On the other way, trypsin and pronase have general applicability for proteolytic detachment of isolated cells [8].
- Dynal (Oslo, Norway) developed a system for detachment of Dynabeads called "DETACHaBEAD". It is a polyclonal Ab that reacts with the Fab fragments of primary MAbs on magnetic beads. DEACHaBEAD effects direct dissociation of the antigen–Ab binding thereby producing cells without Abs remaining on the surface and with unchanged antigen expression [8].
- Synthetic peptides which bind specifically to the antigen binding site of primary Abs have been used by Baxter Healthcare. The peptides compete with the target cell–magnetic particles complexes and enable to obtain target cells with unchanged antigen expression.
- Carbohydrate units on the Fc part of the Abs allow reversible attachment of the Abs to the magnetic particles with immobilized $-B(OH)_3$ groups. After selective isolation of the target cells sorbitol is added which replaces the Ab on the bead [7].
- Biotinylated polyadenylic acid was combined with streptavidin and the resulting polyadenylic acid–streptavidin was conjugated with an Ab–biotin derivative. The immobilized Ab–polyadenylic acid conjugate was separated from the reaction mixture by hybridization with complementary oligonucleotide immobilized on the surface of Dynabeads oligo(dT)25. The immobilized Ab–polyadenylic acid can be released from the carrier, utilizing low-ionic-strength buffers [42].
- A complex primary Ab–DNA linker can be immobilized on magnetic particles and after cell binding the DNA linker can be splitted enzymatically using DNase [41,43].
- *Cryptosporidium* oocysts were successfully released from the immunomagnetic particles by decreasing the pH of the suspension (addition of HCl).
- Reticulocytes and other cells expressing the transferrin receptor (CD71) can be detached by the addition of autologous plasma or normal AB plasma to the rosetted cells. Soluble transferrin receptors in the plasma will displace the target cells transferrin receptors in complex with the primary Ab bound to magnetic particles [8].

Many parameters influence the process of immunomagnetic separation. Incubation time for cell separation is usually 5–60 min while the binding of primary Abs to secondary coated magnetic particles takes usually 30 min or less. In positive isolation, the

Table 5
Examples of isolation of human, animal and plant cells with magnetically labelled antigens

Cells	Type of MS	Magnetic particles	Antigen	Source of cells	Refs.
Antigen-specific T-lymphocytes	D	Dynabeads, M-500 tosyl-activated	Antigenic peptide bound to a major histocompatibility molecule	Murine lymph nodes	[107]
Human chorionic gonadotropin antibody-secreting hybridomas	D	Dynabeads, M-450 tosyl-activated	Human chorionic gonadotropin	Immune splenocytes fused to Sp2 myeloma cells	[108]
Fibrin fragment D dimer antibody-secreting hybridomas	D	Dynabeads, sheep anti-mouse IgG coated with a MAb against antigen	Fibrin fragment, D dimer	Mouse spleen cells fused with X-63 mouse myeloma cells	[109]
Murine lymph node cells	D	Sheep erythrocytes	Amino acid polymer	Murine lymph node	[110]
Tetanus toxin specific B-cells	D	Dynabeads, M-450 tosyl-activated	C fragment of recombinant tetanus toxin	Peripheral blood	[111]

purity of cells generally decreases with time, although the yield increases [8].

The magnetic beads to target cells ratio is also important. At least four coated Dynabeads per estimated target cell is usually enough for separation of cell subsets using both direct and indirect techniques. One to three Dynabeads attached to each target cell provides enough magnetic force to efficiently separate the cells in an appropriate magnetic separator [8].

4.2. Application of immobilized antigens

Antigens immobilized on magnetic beads and colloids can be used for the isolation of Ab expressing or antigen-specific cells. This approach has been successfully used for selection of antigen-specific cells from hybridoma bulk cultures after fusion, hybridoma cell colonies after culturing (selection of cells producing high affinity Abs is possible), hybridoma cell colonies just before subcloning, antigen-specific B cells directly from mouse crude spleen or human peripheral blood or human Ab producing cell lines [8]. Table 5 shows selected examples of this approach.

4.3. Application of immobilized lectins

Many lectins can interact with saccharide residues on the cell surfaces. A typical example of this approach is the application of immobilized *Ulex europaeus* I lectin which binds to terminal L-fucosyl residues present on the surface of human endothelial cells [48]. Lectins have been immobilized on magnetic particles by adsorption [8], by covalent immobilization on tosylactivated beads [49] or biotinylated lectins were coupled to magnetic particles with immobilized streptavidin. Magnetic beads can be released from the isolated cells using a free competing sugar [50]. In Table 6 typical examples of lectin-magnetic separation of prokaryotic cells are

Table 6
Examples of lectin-magnetic separation of microbial cells

Microorganism	Type of MS	Magnetic particles	Lectin	Analyzed sample	Refs.
Brochothrix spp.	D	Dynabeads	*Agaricus bisporus* lectin	Meat samples	[96]
Escherichia coli	D	Tosyl-activated Dynabeads	Concanavalin A	Soil extract	[97]
Listeria monocytogenes	D	Dynabeads M-280 tosyl-activated	*Agaricus bisporus* lectin	Meat samples	[98]
Salmonella enteritidis	D	BioMag particles	*Triticum vulgaris* lectin	Meat samples	[98]
Staphylococcus aureus	D	BioMag particles	*Triticum vulgaris* lectin	Meat samples	[98]

Table 7
Examples of lectin-magnetic separations of human, animal and plant cells

Cells	Type of MS	Magnetic particles	Lectin	Source of cells	Refs.
Duck lymphocytes	D	Tosyl-activated Dynabeads	Phytohaem agglutinin from *Phaseolus vulgaris*	Duck (*Anas platyrhynchos*) blood	[49]
Endothelial cells	D	Tosyl-activated Dynabeads M-450	*Ulex europaeus* agglutinin-1	Primate corpus luteum	[99]
Endothelial cells	D	Tosyl-activated Dynabeads M-450	*Ulex europaeus* I lectin	Human adult rheumatoid and osteoarthritic synovium	[50]
Potato somatic hybrids	I	Streptavidin-magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany)	Biotinylated ConA, *Pisum sativum* lectin or *Wisteria floribunda* lectin	Potatoes	[100]
Microvascular endothelial cells	D	Dynabeads	*Ulex europaeus* I lectin	Adult human dermis	[101]
Plasma cells	D	Dynabeads M-450	Peanut agglutinin	Bone marrow	[102]

shown, while Table 7 shows application of this technique for the separation of eukaryotic cells.

4.4. Application of immobilized oligosaccharides

Magnetic beads and colloids with immobilized oligosaccharides can be used for rapid isolation of specific lectin expressing cells. Biotinylated oligosaccharide–polyacrylamide conjugate was uniformly coated onto magnetic beads with immobilized streptavidin. The polyacrylamide serves as a high-molecular-mass carrier that has low non-specific adsorption and is stable to chemical and proteolytic action. Target cells bound to the magnetic particles can be released using a free competing saccharide structure. The lower association constants for protein–carbohydrate interactions ($K_a=10^3-10^4$) may account for the relatively simple detachment of viable cells [10,51]. A modified procedure, employing glycosphingolipids adsorbed to carboxylated magnetic polystyrene microspheres, was used for the isolation of cells transiently expressing vertebrate lectins [52].

4.5. Application of immobilized phages

Quite recently another approach for the isolation of *Salmonella* cells has been described. A biosorbent consisting of a *Salmonella*-specific bacteriophage (phage) immobilized to a magnetic solid-phase was used for the separation and concentration of *Salmonella* from food materials. The ease of production of phage, high affinity of phage-cell interaction and the ability of phage to infect host cells in heterogeneous environments indicates the potential of such a biosorbent as the basis for a reliable separation system in food microbiological analysis [53].

4.6. Modification of the cell wall with molecular magnetic labels

Both prokaryotic and eukaryotic cells can be magnetically labelled with erbium ions [54], ferritin [2] and magnetoferritin [55]. Erbium ions do not interfere with the typical appearance of the Gram-stained bacteria nor with the antigen recognition by the Ab in the immunochemical evaluations. On average, the reaction of Gram-positive microorganisms is significantly stronger to the magnetic field in the presence of Er^{3+} than the reaction of Gram-negative organisms [54]. Ferritin and magnetoferritin in cationized form have mainly been used for magnetic labelling of lymphocytes [2].

4.7. Other methods of magnetic cell labelling

Magnetotactic bacteria were introduced into granulocytes and monocytes by phagocytosis. The number of phagocytes containing bacterial magnetites (magneto-sensitive cells) became constant after 1.5 h incubation, and viable phagocytes contained about

20–40 cells of magnetotactic bacteria. Granulocytes and monocytes containing bacterial magnetites were magnetically separated from lymphocytes [56].

Entamoeba histolytica takes up 30% of its own volume per hour by fluid-phase pinocytosis and this phenomenon can be used for its magnetic labelling or for the isolation of pinocytic vesicles of defined age [57].

A large number of microorganisms have an affinity to ingest or precipitate (in the form of hydrogen phosphates or sulphides) ion species onto their surfaces. In the case of magnetic ionic species, magnetic separation methods could be applied to remove the ion-loaded organisms from the surroundings. It was shown that the microorganisms developed an appreciable magnetic moment which enabled their removal by magnetic separation. In most cases, starting metal concentration of the order of 10 ppm were reduced to the 1 ppb level. This process will have applications in the mineral processing industry in addition to the treatment of effluents from the nuclear industry and other industrial plants, and in the recovery of precious metals [58,59].

Submicron magnetic particles of γ-Fe_2O_3 adhere to the cells' surfaces of *Saccharomyces cerevisiae*, making the cells magnetic and amenable to magnetic separation. Attachment of the magnetic particles to the yeast surface occurs irrespective of the solution pH and surface charge and is essentially irreversible [60].

4.8. Separation of magnetotactic bacteria and erythrocytes

Magnetotactic bacteria, due to the presence of ferromagnetic material in their cells, can be magnetically separated without any labelling. Both low-intensity magnetic separators and high-gradient magnetic separators can be used for their isolation [61].

Erythrocytes can be separated by the high-gradient magnetic separation technique after conversion of diamagnetic erythrocytes containing oxyferrohemoglobin into paramagnetic red blood cells by the oxidation of the iron atoms in the cell haemoglobin to the ferric state (methaemoglobin) [62]. Erythrocytes, infected by *Plasmodium*, contain paramagnetic hemozoin, that is a component of malarial pigment. Paramagnetic moment of hemozoin is of sufficient magnitude to enable the separation of malaria-infected (hemozoin bearing) erythrocytes from normal erythrocytes and early malaria-infected (non-hemozoin-bearing) erythrocytes using a simple high-gradient magnetic separation device [112].

5. Magnetic separation of cells

A very short overview of possible applications of magnetic separation techniques in microbiology, cell biology and medicine and in parasitology is given in the following sections.

5.1. Applications in microbiology

Immunomagnetic separations (and in some cases also lectin-magnetic separations) have found many applications especially in food, clinical, veterinary and environmental microbiology (see Tables 6 and 8). The basic role of these techniques is the detection of pathogenic microorganisms. Standard microbiology procedures usually require four stages and at least four different growth media; hence the total time from sampling the food to obtaining a result can be measured in days. One of the possibilities for shortening the isolation and detection period is to replace the selective enrichment stage with a non-growth related procedure. This can be achieved by specific magnetic separation of the target organism directly from the sample or the pre-enrichment medium. Isolated cells can than be identified by standard microbiological procedures. IMS is not only faster but also usually gives higher number of positive samples [63]. Also sublethaly injured and dead microbial cells can be isolated using IMS [64]. The principle of IMS of target microorganisms is schematically shown in Fig. 1.

As can be seen in the Table 8, many microorganisms have been isolated by IMS. In most cases specific immunomagnetic particles have been prepared in the laboratory. For the detection of most important microbial pathogens specific immunomagnetic particles are commercially available. They are used for the detection of *Salmonella* (Dynal), *Listeria* (Dynal) and *Escherichia coli* O157 (Dynal and Cardinal, Santa Fe, NM, USA).

Various bacterial antigens such as antigenic deter-

Table 8
Examples of immunomagnetic separation of microbial cells

Microorganism	Type of IMS	Magnetic particles	Primary antibody	Analysed sample	Refs.
Actinobacillus pleuropneumoniae	D	Dynabeads M-280, sheep anti-rabbit IgG	Purified rabbit IgG	Tonsils	[82]
Aeromonas salmonicida	D	Dynabeads M-280, sheep anti-mouse IgG	MAb against lipopoly-saccharides of *A. salmonicida*	Salmon lice *Lepeophtheirus salmonis*	[83]
Bacillus anthracis spores	I	Dynabeads M-280 streptavidin	Biotinylated goat anti-*B. anthracis* spores Ab	Model samples	[46]
Bordatella pertussis	D	Dynabeads M-280, sheep anti-rabbit IgG	Rabbit anti-*B. pertussis* serum	Nasopharyngeal aspirates	[84]
Clostridium difficile	D	Magnisort coated with goat Igs specific for murine IgG and IgM	Murine Mab against *C. difficile*	Stool samples	[85]
Ehrlichia risticii	D	Dynabeads M-280, sheep anti-rabbit IgG	Rabbit anti-*E. risticii*	Horse faeces	[86]
Escherichia coli O157	D	Commercially available Dynabeads anti-*E. coli* O157		Stool samples	[87]
Hellcobacter pylori	D	Dynabeads M-280, sheep anti-rabbit IgG	Polyclonal rabbit anti-*H. pylori* IgG	Stool samples	[88]
Listeria monocytogenes	D	Dynabeads M-280, sheep anti-mouse IgG	Mab against *L. monocytogenes* flagella	Food samples	[89]
Mycobacterium tuberculosis	D	Amine-terminated BioMag	Polyclonal anti-*M. bovis* antibody	Cerebrospinal fluid	[39]
Porphyromonas gingivalis	D	Dynabeads M-280, sheep anti-mouse IgG	Mab against hemagglutinating adhesin	Spiked bacterial suspensions	[90]
Pseudomonas putida	D	Dynabeads M-450, sheep anti-mouse IgG	Murine Mab against *P. putida*	Lake water	[91]
Salmonella sp.	D	Commercially available Dynabeads anti-*Salmonella*		Food samples	[64]
Shigella dysenteriae	D	Dynabeads M-450 uncoated	MAb against O antigens of *S. dysenteriae*	Stool samples	[38]
Streptosporangium fragile spores	I	Dynabeads M-280, sheep anti-rabbit IgG	Purified rabbit polyclonal Abs	Soil	[92]
Thermodesulfobacterium mobile	D	Dynabeads M-280, sheep anti-rabbit IgG	MAb against *T. mobile*	Water samples	[93]
Vibrio parahaemolyticus	I	Dynabeads M-280, sheep anti-rabbit IgG	Rabbit antiserum against *V. parahaemolyticus*	Food samples	[94]
Yersinia enterocolitica O:3	D	BioMag	Abs against *Y. enterocolitica* O:3	Faecal samples	[95]

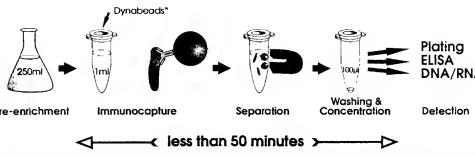

Fig. 1. The principle of immunomagnetic separation of target microorganisms. Reproduced, with permission, from Ref. [122].

minants on the cell wall (O-antigens) or flagellar and fimbrial antigens are usually employed for IMS of bacteria. Both monoclonal and polyclonal Abs are used with success. In various applications both a single, highly specific Ab is immobilized (e.g., detection of *E. coli* O157) or a mixture of monoclonal and polyclonal Abs against various antigenic determinants is immobilized in order to be able to capture various species and strains of one genus (e.g., detection of *Salmonella*). An electron micrograph showing *E. coli* O157 bound to Dynabeads illustrates the IMS effectiveness (Fig. 2).

Target microbial cells isolated using IMS can be characterized using a variety of methods. Magnetic labels usually do not need to be detached from target microbial cells since attachment to immunomagnetic or lectin-magnetic beads has no effect on their growth and cells can continue to multiply if nutritional requirements are provided. Magnetically separated cells can be inoculated on selective agars or liquid nutrient media and tested in the standard way. Magnetically captured cells can also be detected using an impedance technique [65], enzyme-linked immunosorbent assay (ELISA) [66,67] etc.

IMS can be effectively combined with the polymerase chain reaction (PCR). The process combining these two procedures is sometimes abbreviated as MIPA (magnetic immuno PCR assay) [68,69]. The main purpose of IMS is to remove the PCR inhibitory compounds from a sample without loss of sensitivity through dilution. The oligonucleotide primers should be specific either for the target genus (e.g., detection of *Salmonella*) or for the individual strain of interest. Various modifications of PCR for MIPA technique, e.g., nested PCR with two nested pairs of primers in a two-step PCR, are described [70].

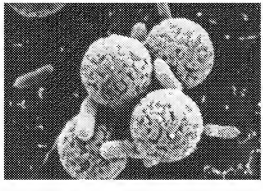

Fig. 2. An electron micrograph showing *E. coli* O157 bound to Dynabeads. Reproduced, with permission, from materials provided by Dynal, Oslo, Norway.

5.2. Applications in cell biology and medicine

There is a large amount of papers describing the application of magnetic separation techniques for the isolation of a variety of eukaryotic cells. Useful information and many examples can be found in several review papers and booklets [6–8]. In the following text only selected applications will be mentioned.

Removal of cancer cells from bone marrow is one of the most important application of IMS. First experiments with the immunomagnetic separation of cancer cells were initiated with Kemshead et al. in 1979. This technique, originally used for removal of neuroblasts from bone marrow intended for autolog-

ous bone marrow transplantation, has been later extended to other tumours. Bone marrow purging in connection with bone marrow transplantation requires a combination of high efficiency in removal of tumor cells and high recovery of hematopoietic stem cells. Usually the indirect method is used, when the cell suspension is most often sensitized with a coctail of monoclonal IgG Abs before mixing with magnetic particles [6,7]. Recently it has been reported that the direct immunomagnetic purging procedure can effectively deplete cancer cells using a mixture of beads with sheep anti-mouse Ab, coated with individual IgG MAbs [7].

From the point of view of prognosis it is necessary to know if there are cancer cells in circulation. Circulating tumour cells are important objects for further research on the metastatic process. Very low number of tumour cells has to be detected in blood. IMS is able to enrich tumour cells before their visualisation but a large number of magnetic beads present in the sample makes their identification by immunocytochemistry difficult. One of the solutions of this problem is the use of gradient centrifugation technique. During this process the excess of magnetic beads, having the highest density, is separated from the tumour cells which even when fully rosetted with beads, have a distinctly lower density. Using this method one may remove >95% of the excess magnetic beads while maintaining >75% of the target cells [7].

Elimination of graft versus host disease (GVHD) in allogenic bone marrow transplantation requires an effective removal of T cells from the bone marrow of the donor. A direct method enabled a 3-log depletion of T cells [71].

Magnetic particles are being increasingly used for isolation of human cell susbsets directly from blood and other cell sources. B lymphocytes [72], endothelial cells [73], granulocytes [74], hematopoietic progenitor cells [75], Langerhans cells [76], leukocytes [8], monocytes [77], natural killer cells [78], reticulocytes [79], T-lymphocytes [8], spermatozoa [80] and many others may serve as examples. Cells from other animal and plant species have been successfully separated, too [8]. Fig. 3 shows T-lymphocytes bound to two Dynabeads M-450 [8].

Not only whole cells, but also cell organelles can be successfully isolated from crude cellular fractions.

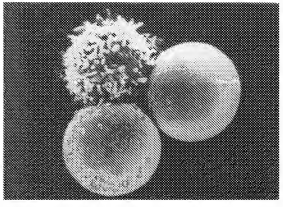

Fig. 3. An electron micrograph showing a T-lymphocyte bound to two Dynabeads M-450. Reproduced, with permission, from Ref. [8].

Dynal has developed Dynabeads M-500 Subcellular which are able to isolate rapidly more than 99% of target organelles [41].

5.3. Applications in parasitology

Up to now, there are not so many examples of application of magnetic separation techniques for the isolation and detection of protozoan parasites. Typical examples of successful applications are shown in Table 9. Protozoan parasites are mainly detected in water and clinical samples.

The occurrence of *Cryptosporidium* outbreaks in drinking water have brought about an increased need for detection at levels necessary to protect human health. Recently two commercially available products for IMS of *Cryptosporidium* have appeared on the market, produced by Dynal and Clearwater Diagnostics, Portland, ME, USA. Both products are used in the "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA (December 1997 Draft)" created by the US Environmental Protection Agency (EPA) [119]. This method was developed to determine *Cryptosporidium* reliably at low concentrations. In very low turbidity samples (clean waters) IMS demonstrated significantly better results than the standard procedures. When water samples were turbid, the recovery efficiency of IMS diminished [81].

Table 9
Examples of immunomagnetic separation of parasites

Parasite	Type of IMS	Magnetic particles	Primary antibody	Analysed sample	Refs.
Cryptosporidium parvum oocysts	D	BioMag with bound goat polyclonal anti-mouse IgM	Monoclonal mouse IgM anti-*Cryptosporidium* oocyst antibody	Water samples	[103]
Cryptosporidium parvum oocysts	I	Dynabeads M-280, streptavidin with bound biotinylated anti-FITC Mab	FITC-labelled Mab	Faeces	[47]
Cryptosporidium parvum oocysts	D	streptavidin MagneSphere paramagnetic particles	Biotinylated purified rabbit IgG against *C. parvum* oocysts	Water samples	[40]
Giardia lamblia cysts	I	Protein A-coated colloidal magnetite particles	Mouse IgG anti-*Giardia* Ab	Water samples	[45]
Plasmodium falciparum	D	Dynabeads M-280, sheep anti-mouse IgG	Three Mabs to merozoite surface protein (MSP1)	Blood	[104]
Toxoplasma gondii, bradyzoite form	I	Rat anti-mouse IgG1 magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany)	Mouse bradyzoite-specific Mab	Mixture of bradyzoites and tachyzoites	[105]
Trypanosoma brucei (transgenic cells expressing glutamic acid/alanine-rich protein)	I	Dynabeads coated with sheep anti-rat IgG	Polyclonal rat anti-GARP antiserum	Model suspension	[106]

6. Combination of magnetic, chromatographic and electromigration methods

Target cells pre-separated by magnetic separation techniques can be used for further analysis of the cell content using chromatographic and electromigration methods.

Magnetic cell separation can be effectively combined with PCR and the amplified material analyzed with gel electrophoresis. In one of the approaches target microbial cells isolated from food and clinical samples were lysed and after centrifugation the supernatant containing DNA was added into PCR reaction mixture. The PCR products were analyzed with gel electrophoresis [120]. Another paper describes the accurate and rapid determination of the origin of haemopoietic cells following allogeneic bone marrow or peripheral blood stem cell transplantation. This procedure is based on the immunomagnetic capture of white cells combined with microsatellite polymerase chain reaction and resolution of products by polyacrylamide gel electrophoresis [121]. In the second approach specific leukocytes were obtained using immunomagnetic separation and after cell lysis poly A+mRNA was isolated with magnetic particles containing immobilized oligo deoxythymidine. Isolated mRNA was used to synthesize cDNA which was further amplified using PCR and then analyzed by gel electrophoresis [122].

Superparamagnetic nanoparticles with bound affinity ligands, which were used to label the target cells, were characterized using an electrophoretic procedure. The surface charge modifications of nanoparticles, caused by ligand coupling, were monitored by measuring their electrophoretic mobilities using laser-Doppler velocimetry. Particle electrophoretic mobility changes correlated well with the amount of ligand fixed on the particles [126].

Also chromatographic procedures can be combined with magnetic cell separations. To determine which mice splenic cell type was capable of metabolizing benzo[*a*]pyrene (B[*a*]P) the splenocytes were separated using combination of methods employing also negative IMS. The isolated splenic cell populations were incubated with [3H]B[*a*]P for 24 h. High-performance liquid chromatography was used to separate and quantitate the

B[*a*]P metabolites generated by the splenic cells [123].

Magnetically stabilized fluidized bed chromatography was used to separate yeast cells and cell debris from suspensions [116,124]. Alternatively MSFB technology was used to construct a novel bioreactor which enables plant cells immobilized in calcium alginate beads to be cultivated continuously [125].

7. Conclusions and future perspectives

Magnetic separation of cells has shown its usefulness in many applications where other procedures failed. This technique exhibits high specificity, can be easily scaled-up, many ready-to-use products are available and the basic equipment for standard work is relatively inexpensive.

Nowadays immunomagnetic separation is the standard procedure and the same situation can be expected in the future. Nevertheless, the importance of other specific affinity ligands interacting with specific cell surface structures, such as lectins, carbohydrates, antigens or phages will be increasing.

Magnetic separation techniques are compatible with majority of other techniques used in biosciences. One of the advantages of this system, i.e., the possibility of selective separation of target cells together with removal of interfering substances is valuable for the PCR and related techniques. Combination of these two principles is especially important in food, clinical a environmental microbiology, cell biology, medicine and parasitology.

Separation of target cells using immunomagnetic and similar particles is the main area where magnetic techniques showed their potential. Nevertheless, magnetic particles can label the target cells and tissues in order to transport drugs, or to enable radiotherapy or hyperthermia [24]. Current and future developments will use magnetic carriers more and more for combination therapies [113].

Magnetic separation techniques can be automated and miniaturized. On the other hand, large-scale applications are also possible. The scope of possible applications of the discussed techniques is very broad and many new procedures in various fields of biosciences and biotechnologies will undoubtedly be developed in the near future.

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